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Integrin activation.

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Journal

BMB reports, 47(12)

ISSN

1976-6696

Author

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Publication Date

2014-12-01

DOI

10.5483/bmbrep.2014.47.12.241

Peer reviewed

Invited Mini Review

Integrin activation

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Integrin-mediated cell adhesion is important for development, immune responses, hemostasis and wound healing. Integrins also function as signal transducing receptors that can control intracellular pathways that regulate cell survival, proliferation, and cell fate. Conversely, cells can modulate the affinity of integrins for their ligands a process operationally defined as integrin activation. Analysis of activation of integrins has now provided a detailed molecular understanding of this unique form of “inside-out” signal transduction and revealed new paradigms of how transmembrane domains (TMD) can transmit long range allosteric changes in transmembrane proteins. Here, we will review how talin and mediates integrin activation and how the integrin TMD can transmit these inside out signals. [BMB Reports 2014; 47(12): 655-659]

INTRODUCTION

Integrins, heterodimeric type I transmembrane proteins consisting of α and β subunits, are a major class of receptors involved in adhesive events that control development and lead to pathologies such as cancer and thrombosis. Eighteen integrin α subunits and 8 β subunits heterodimerize to form 24 different integrins (1). Each subunit contains a single transmembrane domain (TMD) and a short cytoplasmic tail. Besides mediating cell adhesion, integrins transmit signals across the plasma membrane that regulate cell migration, cell survival and growth (2). Conversely, signals from inside cells can increase the binding of integrin extracellular domains to ligands, a process operationally defined as integrin activation. Integrin activation encompasses both changes in affinity of individual integrins due to conformational changes and avidity increases due to integrin clustering (3-5). Precise regulation of integrin activation is particularly important in controlling platelet aggregation through integrin α IIb β 3 (6). Rapid activation of this integrin at the site of a wound is required for hemostasis (7);

conversely inappropriate activation of α IIb β 3 can cause a platelet thrombus to occlude a blood vessel resulting in myocardial infarction or stroke. Here we will discuss recent progress in understanding how integrins are activated.

INTEGRIN TMD-THE CONDUIT FOR ALLOSTERIC REARRANGEMENTS

Changes in the conformation of integrin extracellular domains are responsible for the changes in integrin monomer affinity. These conformational changes have been the subject of several excellent reviews (3, 8-12) and will not be discussed here. Similarly, clustering of integrins can enhance the binding of multivalent ligands and kindlins have recently emerged as major players in clustering (13). The capacity of intracellular signals to change the conformation of the extracellular domain requires a remarkable transmembrane allosteric change, a change that must traverse the integrin TMD. Truncation of the integrins at the C-termini of extracellular domains results in constitutively active integrins (14), indicating that TMDs and cytoplasmic tails limit the activation state of integrins. Furthermore, many activating mutations, map to the α or β TMD (15-18). Heterodimeric interactions between α and β TMDs and cytoplasmic tails have been observed by co-immunoprecipitation (19), cysteine crosslinking (20, 21) and by NMR (22) in phospholipid bicelles, but not in detergent micelles (23). Importantly, mutations in TMDs that activate integrins invariably inhibit α and β TMD interactions (19). Thus, physiological integrin activation is likely to require that intracellular signals disrupt integrin $\alpha\beta$ TMD interactions.

The structure of the α IIb β 3 TMD complex in a phospholipid bicelle (22) revealed the basis of association of the α and β through two interaction interfaces. The α IIb TMD helix is short, straight and broken at Gly⁹⁹¹, the first residue of the highly-conserved Gly-Phe-Phe-Lys-Arg (GFFKR) motif in the membrane proximal region of the α subunits. The two Phe residues of the α IIb GFFKR motif do not form a continuous helix but instead make a sharp turn toward β 3 (Fig. 1). In this way, the hydrophobic side chains of those residues reside in the hydrophobic core of the lipid bilayer and stack against hydrophobic residues in the β 3 TMD, particularly Trp⁷¹⁵ and Ile⁷¹⁹. The turning of the membrane-proximal region of α IIb also permits the long-predicted (24) electrostatic interaction between α IIb Arg⁹⁹⁵ and β 3 Asp⁷²³ by placing those residues in proximity (Fig. 1). The structure at the inner membrane interface is

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<http://dx.doi.org/10.5483/BMBRep.2014.47.12.241>

Received 7 November 2014

Keywords: Cell adhesion, Integrin, Nanodisc, Signal transduction, Talin, Transmembrane domain

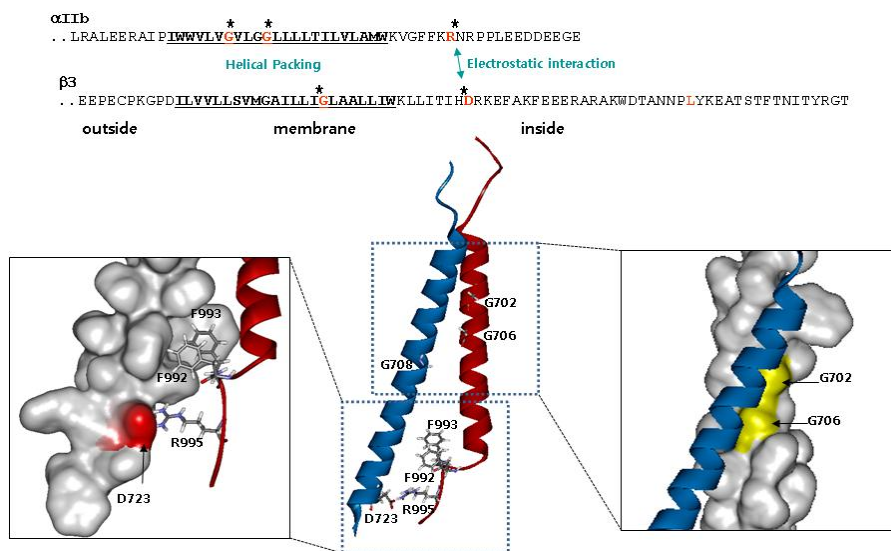


Fig. 1. Structure of integrin α IIb β 3 TMD (ribbon view; α IIb in red and β 3 in blue. From PDB 2K9J) showing the two interaction interfaces. Right, outer membrane clasp (OMC) illustrating the helical packing involving α IIb Gly 702 and 706. Left, inner membrane clasp (IMC) showing the electrostatic interaction between α IIb Arg⁹⁹⁵ and β 3 Asp⁷²³. Also depicted are the hydrophobic interactions of α IIb Phe^{992,993} with the β 3 TMD. Adapted from reference (22).

unique to and likely conserved in integrins and is termed the inner membrane clasp (IMC) (22). The second interface involves helical packing centered on β 3 Gly⁷⁰⁸ and α IIb G⁹⁷²XXXG⁹⁷⁶ motif at the outer membrane region and is termed the outer membrane clasp (OMC) (Fig. 1). Integrin β 3 TMD makes a long and continuous helix with a 25° tilting angle to enable the multipoint interactions with α IIb and accommodate the extra hydrophobic residues in the β 3 TMD.

As noted above, an important feature of the structure of the α IIb β 3 TMD dimer is that the helical β TMD must be precisely tilted to maintain simultaneous formation of the OMC and IMC. Precise tilt is maintained via β 3 Lys⁷¹⁶ whose alpha carbon resides in the hydrophobic region of the lipid bilayer but its positively charged ϵ -NH₃⁺ is predicted to snorkel into the negatively charged phosphate head group region (25). Mutation of Lys⁷¹⁶ any residue other than Arg (which also contains a snorkeling basic side chain) reduces α - β TMD interactions and dramatically increases integrin activation (25). The effects of Lys⁷¹⁶ mutation can be ameliorated by breaking the continuous β TMD helix into two halves by introduction of a Pro mutation, introduces a flexible hinge that partially decouples the tilting angles of inner and outer helices favoring simultaneous formation of OMC and IMC (25).

TALIN "TILTS" THE INTEGRIN β TMD TO INDUCE ACTIVATION

Talin regulates integrin affinity and provides a mechanical link between integrins and the actin cytoskeleton. Talin comprises a 50-kDa N-terminal FERM domain (talin head domain or THD) that contains a high-affinity binding site for integrin β tails and a 220-kDa rod domain that contains multiple binding

sites for actin and vinculin (26). The THD is further divided into F0, F1, F2 and F3 subdomains (26, 27). The F3 subdomain, contains the major integrin integrin β tail binding site (28, 29). The essential role of talin in regulating integrin affinity has been well documented in model cells (29-32), transgenic mice (33-36) and reconstituted systems with purified proteins (37). In *in vitro* systems, recombinant THD alone is sufficient to activate α IIb β 3 reconstituted in either liposomes or phospholipid nanodiscs, and activation is associated with a shift towards an α IIb β 3 extended conformation (37).

We now have considerable insight into how talin induces this allosteric rearrangement in integrins. Talin binds to two sites on integrin β tails: a strong binding site centered around the first NPxY motif that contributes most of the binding free energy and a weaker membrane proximal (MP) binding site (38). In addition, THD also binds to negatively charged phospholipids via positively-charged residues (38-40). The weak interaction with the MP region has two important effects: 1) it brings talin Lys³²⁴ close to Asp⁷²³ of the β 3 tail, thus competing for the Arg⁹⁹⁵-Asp⁷²³ electrostatic interactions in IMC (40); 2) it stabilizes α -helical structure of the β MP region and to form a continuous helix with the β 3 TMD (38, 40). As the simultaneous interaction with integrin β tails and phospholipids, can change the tilt angle of the β 3 MP tail and thus of the contiguous β 3 TMD (Fig. 2) (41). Such talin-induced motion was demonstrated by increased fluorescence of solvatochromic dyes attached to the N- or C-termini of the β 3 TMD in the presence of THD (41) and is further supported by molecular dynamic simulations (42). The change in tilting angle destabilizes α - β TMD interactions and shifts the equilibrium towards an activated integrin conformation. In further support of this model, introducing a flexible proline kink in the middle of the β 3 TMD blocks THD-induced tilting of the outer membrane seg-

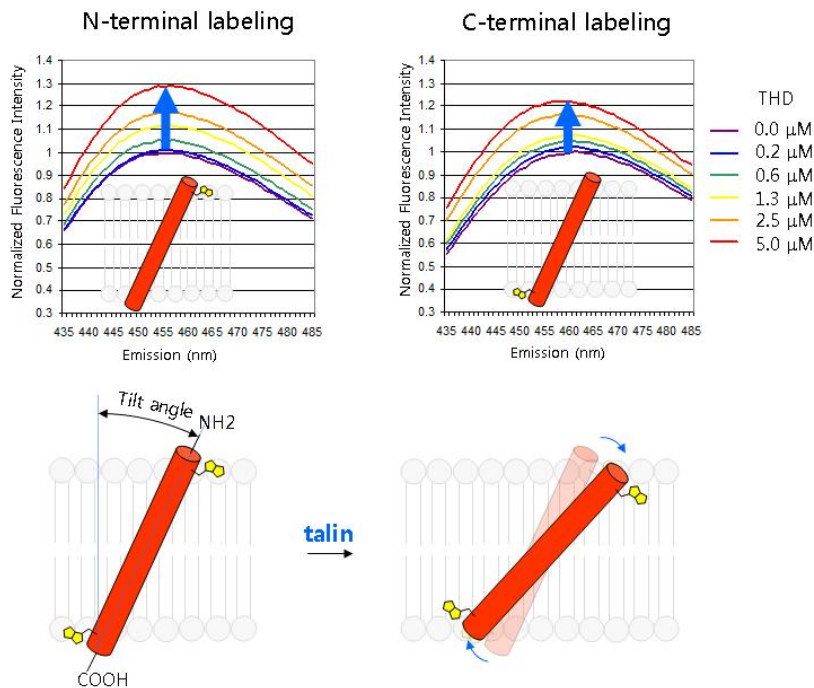


Fig. 2. Talin changes the topology of the $\beta 3$ TMD. A peptide containing the $\beta 3$ TMD and cytoplasmic domain was labeled with environment sensitive bimaes at the outer edge of the TMD (N terminal labeling, Leu⁶⁹⁴) or at the TMD cytosol interface (C-terminal labeling, Ile⁷²¹). The peptides were individually embedded in phospholipid nanodiscs and increasing concentrations of talin head domain (THD) were added and bimaes emission spectra were recorded. The increased fluorescence indicates that both sides of the $\beta 3$ TMD were in a less polar environment suggesting that THD increased the tilting of the $\beta 3$ TMD. Adapted from reference (41).

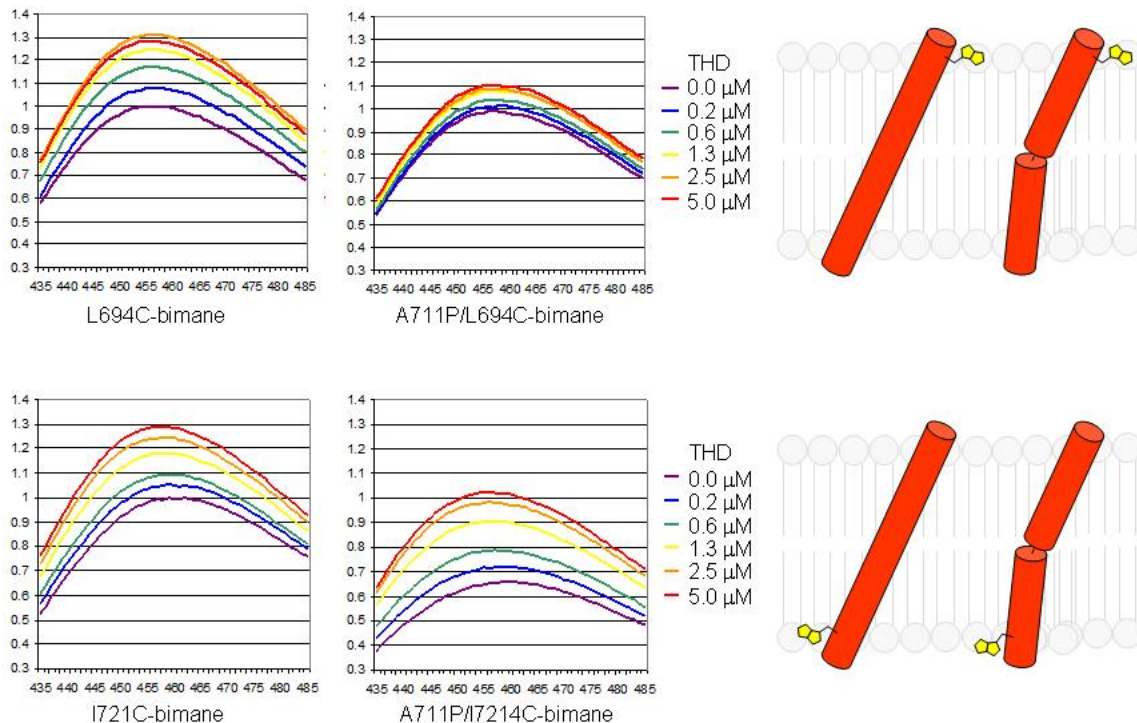


Fig. 3. A proline kink prevents transmission of altered tilt across the $\beta 3$ TMD. In the left two panels The experimental design was identical to that in Fig. 2 and depicts the talin-induced increased embedding at both the inner (where THD binds) and outer edges of the TMD. Introduction of a flexible kink by $\beta 3$ (A711P) mutation (right two panels) prevents the transmission of increased embedding of the inner TMD to the outer region. Adapted from reference (41).

ment without blocking tilting of the inner membrane segment (Fig. 3). Integrins bearing this mutation are remarkably resistant to talin induced integrin activation (41).

SUMMARY AND CONCLUSIONS

Integrin activation was first observed in 1978 in integrin $\alpha\text{IIb}\beta 3$ and it has proved to be a conserved property of $\beta 1$, $\beta 2$, and $\beta 3$ integrins. As summarized here, our understanding of this unique form of transmembrane signal transduction has moved from a black box in which agonists, such as thrombin, caused a change in the affinity of integrin $\alpha\text{IIb}\beta 3$. Today, cell biological and reverse genetic experiments have verified that talin binding to the integrin β cytoplasmic domain is a final common step in activation. Structural studies have revealed how two binding interfaces of talin with the integrin in combination with talin membrane binding sites can effect this form of transmembrane allostery. Studies have also revealed unique features of the heterodimeric integrin TMD that form a stable yet dynamic $\alpha\beta$ TMD interaction that enables transmission of the activation signal across the phospholipid bilayer. The lessons learned in studying integrin transmembrane signaling, such as the importance of snorkeling basic residues in maintaining TMD topology, are likely to pertain to other examples of transmembrane signaling through transmembrane receptors.

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